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Sufficient Evidence for Lymphatics in the Developing and Adult Human Choroid?

We read with interest the recent article by Koina et al.¹ suggesting evidence for the presence of lymphatic vessels in the developing and adult human choroid. However, this study does not meet the recently published consensus criteria on the immunohistochemical detection of ocular lymphatic vessels,² and therefore, in our opinion, requires critical revision.

First, appropriate positive and unequivocal negative controls are not presented in the study of Koina et al. In particular, when describing novel anatomical structures for the first time, and in order to change an existing dogma, a detailed documentation of blood and lymphatic vessel detection in the control tissue is mandatory. The provided supplementary data do not fulfill these criteria.

Second, the immunohistochemical marker panel used is critical. Endomucin does not represent an established lymphatic marker,^{3,4} but is rather expressed by “endothelial cells along the whole vascular tree including lymphatic vessels.”⁵ Thus, an unequivocal discrimination between blood and lymphatic vessels is impossible with this marker. A further discrepancy is the use of the transcription factor prospero-related homeobox gene-1 (Prox-1) as an extranuclear lymphatic endothelial precursor marker. Although reports of the extranuclear presence of PROX-1 in cell types other than lymphatic endothelium exist,^{6–8} PROX-1 clearly shows a nuclear expression in lymphatic endothelia in human,⁹ as well as mouse¹⁰ and avian,¹¹ embryos, retaining its nuclear localization into adulthood.^{12–14} On the other hand, it is not clear why lymphatic endothelial surface markers, such as podoplanin, lymphatic vascular endothelial-specific hyaluronic acid receptor-1 (LYVE-1), and the vascular endothelial marker CD34 display nuclear expression in this study. Additionally, the only lymphatic endothelial cell marker used in whole mounts is VEGFR-3, which is also expressed in fenestrated blood vessels, and, as such, also in the choriocapillaris.^{15,16} Morphologically, the supposed lymphatic VEGFR-3-positive vessels are indistinguishable from the honeycomb-like lobular pattern of the choriocapillaris.¹⁷

Furthermore, the study of Koina et al. includes a blatant inconsistency in the use and documentation of immunohistochemical markers between fetal and adult eyes. Although one has to acknowledge that certain lymphatic markers might be expressed during embryogenesis, this pattern easily changes during maturation.¹⁸ Therefore, such an approach would require extensive comparison of the same markers in different ages, thus representing an extensive survey in its own right. However, this is not the case in the study of Koina et al.

Third, the ultrastructural study would be greatly strengthened by immunoelectron microscopy. Indeed, anchoring filaments with a diameter of 40 to 100 Å—becoming readily identifiable only at magnifications of 40,000× to 50,000×—are present in lymphatics,¹⁹ but could be easily present in the choroid as well without any association to lymphatic vessels,^{20–22} particularly in aged eyes with typical alterations of the extracellular matrix. For this purpose, as well as for ruling out Weibel-Palade bodies, serial ultrathin sectioning with appropriate labeling would be necessary. Despite possible postmortem tissue alterations, numerous previous studies successfully applied different detection systems for ultrastructural investigations using ocular human donor tissue.^{23–29} A limited use of immunomarkers for these investigations, as claimed, seems therefore not justified.

In regard to the above-mentioned criticisms, the evidence presented in the study of Koina et al. does not justify the hypothesized paradigm shift that functional lymphatic vessels are present in the human choroid. Rather, the findings of Koina et al. confirm previous reports of net-like structures with a “pseudo-vessel” appearance in the human choroid endowed with lymphatic vascular precursor cells (represented as LYVE-1⁺ macrophages).²⁵ Those “atypical” lymphatic-like cells (i.e., endothelial cells with divergent or uncommon immunohistochemical phenotypes) may also exist in other parts of the eye. For example, the endothelial cells of Schlemm’s canal display many, but not all, features of terminally differentiated lymphatic endothelial cells, including responsiveness to VEGF-C-induced lymphangiogenesis.³⁰

In closing, we acknowledge that the work of Koina et al. is a further contribution to our understanding of the choroid, but although the existence of lymphatics in the human choroid cannot be ruled out per se, because of the aforementioned points and the sheer volume of evidence to date, we maintain that the inner human eye and in particular the choroid should still be considered an immune-privileged site devoid of lymphatic vessels. Further unequivocal evidence of “typical lymphatic vessels” in the human choroid is still missing.

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References

- Koina ME, Baxter L, Adamson SJ, et al. Evidence for lymphatics in the developing and adult human choroid. *Invest Ophthalmol Vis Sci.* 2015;56:1310–1327.
- Schroedl F, Kaser-Eichberger A, Schlereth SL, et al. Consensus statement on the immunohistochemical detection of ocular lymphatic vessels. *Invest Ophthalmol Vis Sci.* 2014;55:6440–6442.
- Baluk P, McDonald DM. Markers for microscopic imaging of lymphangiogenesis and angiogenesis. *Ann N Y Acad Sci.* 2008;1131:1–12.
- Karunamuni G, Yang K, Doughman YQ, et al. Expression of lymphatic markers during avian and mouse cardiogenesis. *Anat Rec (Hoboken).* 2010;293:259–270.
- Samulowitz U, Kuhn A, Brachtendorf G, et al. Human endomucin: distribution pattern, expression on high endothelial venules, and decoration with the MECA-79 epitope. *Am J Pathol.* 2002;160:1669–1681.
- Demidenko Z, Badenhorst P, Jones T, Bi X, Mortin MA. Regulated nuclear export of the homeodomain transcription factor Prospero. *Development.* 2001;128:1359–1367.
- Duncan MK, Cui W, Oh DJ, Tomarev SI. Prox1 is differentially localized during lens development. *Mech Dev.* 2002;112:195–198.
- Truman LA, Bentley KL, Smith EC, et al. Prox1 lymphatic vessel receptor mice reveal Prox1 expression in the adrenal medulla, megakaryocytes, and platelets. *Am J Pathol.* 2012;180:1715–1725.
- Rodriguez-Niedenführ M, Papoutsis M, Christ B, et al. Prox1 is a marker of ectodermal placodes, endodermal compartments, lymphatic endothelium and lymphangioblasts. *Anat Embryol (Berl).* 2001;204:399–406.
- Buttler K, Kreysing A, von Kaisenberg CS, et al. Mesenchymal cells with leukocyte and lymphendothelial characteristics in murine embryos. *Dev Dyn.* 2006;235:1554–1562.
- Wilting J, Becker J. Two endothelial cell lines derived from the somite. *Anat Embryol (Berl).* 2006;211Suppl 1:57–63.
- Petrova TV, Mäkinen T, Mäkelä TP, et al. Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J.* 2002;21:4593–4599.
- Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. *Cell.* 1999;98:769–778.
- Wilting J, Papoutsis M, Christ B, et al. The transcription factor Prox1 is a marker for lymphatic endothelial cells in normal and diseased human tissues. *FASEB J.* 2002;16:1271–1273.
- Partanen TA, Arola J, Saaristo A, et al. VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues. *FASEB J.* 2000;14:2087–2096.
- Blaauwgeers HG, Holtkamp GM, Rutten H, et al. Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a trophic paracrine relation. *Am J Pathol.* 1999;155:421–428.
- Bhutto IA, Luttly GA. The vasculature of choroid. In: Schepers D, D'Amore PA, eds. *Encyclopedia of Microvasculatures*. San Diego: Elsevier; 2004.
- Yang Y, Oliver G. Development of the mammalian lymphatic vasculature. *J Clin Invest.* 2014;124:888–897.
- Leak LV, Burke JF. Ultrastructural studies on the lymphatic anchoring filaments. *J Cell Biol.* 1968;36:129–149.
- May CA, Neuhuber W, Lütjen-Drecoll E. Immunohistochemical classification and functional morphology of human choroidal ganglion cells. *Invest Ophthalmol Vis Sci.* 2004;45:361–367.
- Spitznas M. The fine structure of the chorioretinal border tissues of the adult human eye. *Adv Ophthalmol.* 1974;28:78–174.
- Sellheyer K, Spitznas M. Morphology of the developing choroidal vasculature in the human fetus. *Graefes Arch Clin Exp Ophthalmol.* 1988;226:461–467.
- Birke K, Lütjen-Drecoll E, Kerjaschki D, Birke MT. Expression of podoplanin and other lymphatic markers in the human anterior eye segment. *Invest Ophthalmol Vis Sci.* 2010;51:344–354.
- Hiscott P, Seitz B, Schlötzer-Schrehardt U, Naumann GO. Immunolocalisation of thrombospondin 1 in human, bovine and rabbit cornea. *Cell Tissue Res.* 1997;289:307–310.
- Schroedl F, Brehmer A, Neuhuber WL, Kruse FE, May CA, Cursiefen C. The normal human choroid is endowed with a significant number of lymphatic vessel endothelial hyaluronate receptor 1 (LYVE-1)-positive macrophages. *Invest Ophthalmol Vis Sci.* 2008;49:5222–5229.
- Pena JD, Netland PA, Vidal I, Dorr DA, Rasky A, Hernandez MR. Elastosis of the lamina cribrosa in glaucomatous optic neuropathy. *Exp Eye Res.* 1998;67:517–524.
- Provis JM, Penfold PL, Edwards AJ, van Driel D. Human retinal microglia: expression of immune markers and relationship to the glia limitans. *Glia.* 1995;14:243–256.
- Kolb H, Zhang L, Dekorver L. Differential staining of neurons in the human retina with antibodies to protein kinase C isozymes. *Vis Neurosci.* 1993;10:341–351.
- Cursiefen C, Schlötzer-Schrehardt U, Küchle M, et al. Lymphatic vessels in vascularized human corneas: immunohistochemical investigation using LYVE-1 and podoplanin. *Invest Ophthalmol Vis Sci.* 2002;43:2127–2135.
- Aspelund A, Tammela T, Anttila S, et al. The Schlemm's canal is a VEGF-C/VEGFR-3-responsive lymphatic-like vessel. *J Clin Invest.* 2014;124:3975–3986.

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